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Original

Microwave Irradiation of Lignocellulosic Materials

VIII. Microwave Irradiation of the Neutral Fraction (C-I-M) of Pine Björkman LCC

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Abstract——Effect of microwave irradiation on the partially acetylated galactoglucomannan bearing a small amount of lignin (C-I-M) isolated from pine Björkman LCC was investigated. When the native C-I-M was heated above 180°C by microwave irradiation in the presence of water, its carbohydrate portion was hydrolyzed into oligosaccharides having d.p. of 2–5 and monosaccharides. The degree of depolymerization of carbohydrates strongly depended upon the heating temperature and did not reach a maximum below 237°C, at which the reducing sugar content attained to 45.7%. The lignin molecules precipitated during microwave irradiation and a substancial amount of their β-*O*-4 linkages were splitted. The lignin-carbohydrate bondings were also splitted at the heating temperature above 230°C. The hydrolysis of the carbohydrate portion of C-I-M was found to be remarkably enhanced by addition of 0.5% acetic acid during microwave irradiation. In this case, the reducing sugar production showed a maximum (74.2%) at about 210°C. Acetic acid was an excellent reagent for enhancement of the extent of depolymerization of galactoglucomannan in C-I-M.

1. Introduction

Microwave irradiation of lignocellulosic materials in the presence of water has shown to enhance enzymatic susceptibility of cellulose[1~4]. For clarifying the reason of this new phenomenone, it seems neccessary to study the mechanistic action of microwave irradiation on the constituents of lignocellulosic materials. It has been previously shown that crystallinity of cellulose remains stable against a short time treatment (<10 min) with microwave energy below 230°C[1,2]. In contrast, hemicelluloses and lignin which encrust cellulose were degraded during microwave irradiation above 180–190°C[1~4]. Hence, degradation of these matrix constituents is suggested to increase the surface area of cellulose available for enzymatic attack. However, oversimplification of the effects of microwave irradiation of lignocellulosic materials should be avoided, since their cell-walls are composed of different kinds of constituents whose three dimentional arrangements have not been fully elucidated

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yet.

In order to further clarify the effects of microwave irradiation on lignocellulosic materials, it seems neccessary to heat the purified respective constituents separately by microwave irradiation. In the previous papers[5,6], micro-crystalline celluloses were selected for the initial step of this line of experiments.

In this paper, the well-known neutral acetyl galactoglucomannan bearing a small amount of lignin (C-I-M)[7] isolated from pine Björkman LCC[8] was selected as a model of softwood native hemicelluolse. The methods were the same as those employed for microwave irradiation of the native pine wood[1]. The effects of addition of acetic acid during microwave irradiation on the depolymerization of C-I-M are also described.

2. Materials and Methods

2.1 Materials

Sephadex G-100, DEAE-Sephadex A-50 and Blue Dextran 2000 were supplied by Pharmacia Fine Chemicals. Pullulans having known molecular weights (Shodex Standard P-82) was obtained from Showa Denko, Co., Ltd. Commercial silica gel thin-layer chromatographic plates (0.5 mm thickness, 5715) were obtained from E. Merk, Darmstadt. Björkman lignin-carbohydrate complex (LCC) was extracted with *N*, *N*-dimethylformamide from the sapwood of pine (Akamatsu, *Pinus densiflora* S. and Z.) obtained at the Kamigamo Experimental Forest of Kyoto University[7,8]. The neutral acetyl galactoglucomannan bearing a small amount of lignin (C-I-M) was separated from acidic components of Björkman LCC by ion-exchange chromatography on DEAE-Sephadex A-50 (carbonate form)[8]. The gross chemical properties of C-I-M were as follows; 6.9% lignin, 6.8% acetyl, molar ratios of D-galactose, D-glucose and D-mannose of 5.5 : 21.7 : 72.8, wieght average molecular weight of 1.2×10^4, and $[\alpha]_D^{20}$ −27.8 (*c* 0.5, water). Milled wood lignin (MWL) was prepared from the same pine wood that was used for isolation of C-I-M by the Björkman's method[9]. All other reagents used were analytical reagent grade.

2.2 Microwave irradiation

One gram of C-I-M was dissolved in 14 ml of distilled water placed in glass vessels of 50 ml capacity (Type A-3-L_2, Taiatsu Glass Industry, Co., Ltd.) which were sealed with stainless stoppers after degasification of the solution by aspiration. For analysis of the effects of the addition of acetic acid on C-I-M during microwave irradiation, 0.5% (v/v) aqueous acetic acid solution was used instead of distilled water. Microwave irradiation at 2,450±50 MHz and 2.4 kW (Toshiba Model TMB 3210) was performed as previously described with monitoring the tempera-

ture by infra-red thermometer (Model 6T16, San-ei Sokki Industry, Co., Ltd.)[1,2].

2.3 Evaluation of microwave irradiation

Each irradiated sample was rapidly cooled to room temperature by using an electric fan and pH of the solutions was determined. Each sample was then centrifuged at 1.4×10^4 g for 20 min at 20°C. The water-insoluble material was thoroughly washed with distilled water and weighed to determine the water-insoluble components. The supernatant and washings were combined to make 200 ml with distilled water. The reducing sugars in this solution was determined by the dinitrosalicylic acid (DNS) method using D-glucose as a standard[10]. The molecular weight distribution of the water-soluble components was analyzed by gel filtration chromatography (GFC) on a column (45×1.4 cm) of Sephadex G-100 calibrated against pullulan standards at room temperature using 0.025 M sodium phosphate buffer as an eluent. The neutral oligosaccharide analysis was performed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). HPLC was performed on a Toyo Soda HLC-803B with a Toyo Soda RI-8 detector and a column (25×0.46 cm) of Finepak SIL NH_2 (Japan Spectroscopic Co., Ltd.). The column was eluted with 55% aqueous acetonitrile at room temperature. TLC was developed with 1-butanol-2-propanol-water (3 : 12 : 4, v/v) and reducing sugars detected with *p*-anisidine hydrochloride and 10% sulfuric acid. Infra red (IR) spectra were determined for KBr discs, using a Hitachi model 260–30 spectrophotometer. ^{13}C-Nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-200 NMR spectrometer (50.3 MHz) in an acetone-d_6-deuterium oxide mixture (9 : 1, v/v) at 22–23°C. Chemical shifts in ppm are given as the values relative to that of internal tetramethylsilane. The other experimental methods and instrumentations were the same as those previously described[1,2].

Results and Discussion

3.1 Autohydrolysis of acetyl galactoglucomannan bearing a small amount of lignin (C-I-M)

C-I-M was treated with microwave energy in the presence of distilled water and the temperature dependences of the pH values and the formations of reducing sugars and water-insoluble components were determined (Fig. 1). The time needed to heat C-I-M to about 230°C was 6–7 min, in good agreement with those for agricultural lignocellulosic wastes[2] and that for the sapwood of Akamatsu[1] from which C-I-M was isolated. The pH values of the irradiated solution rapidly dropped down above 160°C to become about 3.4 at 237°C. The acidity increased with a decrease in pH values to attain 1.0 meq at 237°C (Table 1), which corresponded

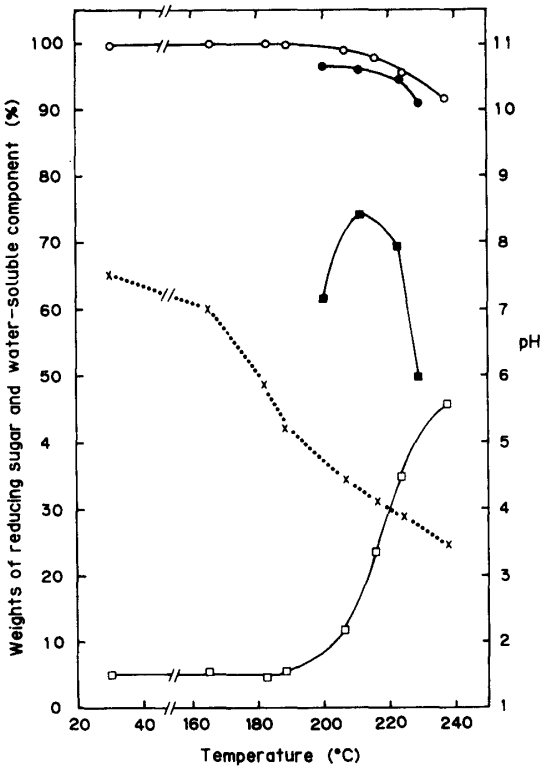

Fig. 1. Changes of pine C-I-M by microwave irradiation of pine C-I-M. Symbols: ○, ●, weights of water-soluble components; □, ■, reducing sugar productions; ×, pH. Open symbols are for microwave irradiation in water, closed for microwave irradiation in the presence of 0.5% acetic acid.

Table 1. Production of acid, molecular weight of the water-soluble fractions, and sugar content in the precipitates formed by microwave irradiation of pine C-I-M

Temperature (°C)	Acidity (meq)	$\bar{M}_w$[a)]	Sugar content in the precipitates formed during microwave irradiation[b)] (%)
169	0.07	9,000	n.d.[c)] (n.d.)[c)]
207	0.18	4,200	23.0 (0.5)
216	0.34	3,250	9.2 (0.3)
225	0.52	2,650	7.9 (0.3)
237	1.10	220	1.2 (0.1)

a) Mean weight average molecular weights of the carbohydrates eluted from Sephadex G-100 (Fig. 2).

b) Based on the dry matter of the precipitates. Values in the parentheses denote the sugar contents in the precipitates calculated based on the dry matter of the original C-I-M.

c) Not determined.

to that of 0.07% aqueous acetic acid solution. Assuming that all of the esterified acetic acid in C-I-M was released at this temperature, the concentration of acetic acid should theoretically be 0.07% in good agreement with the observed vaules. In addition, GLC analysis[1] confirmed that the major organic acid produced was acetic acid. Based on these results, it may be concluded that all of the esterified acetic acid in the native C-I-M was de-esterified during microwave irradiation.

The reducing sugar content did not change below 180°C, above which it rapidly increased with increase in temperature and did not show a maximum up to 237°C, the highest temperature attained in the present experiments (Fig. 1). The highest production of reducing sugars obtained at 237°C was 45.7%. The molecular weight distribution of the water-soluble components was analyzed by GFC (Fig. 2). One broad peak originally observed in the native C-I-M retarded as the temperature increased. The elution profiles of carbohydrates overlapped with those of water-soluble lignin below 200°C, above which, however, the peak of lignin appeared at retard and eluted behind carbohydrates, indicating that the lignin-carbohydrate

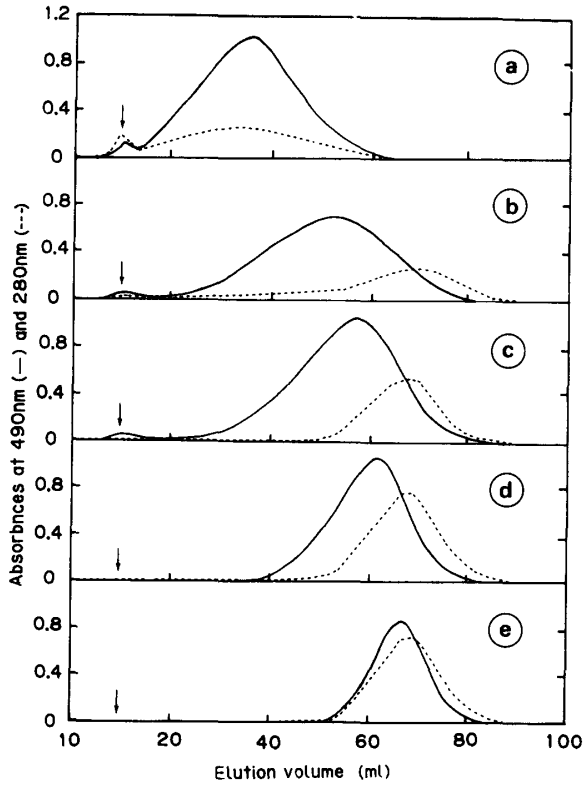

Fig. 2. Gel filtration chromatography of the water-soluble components on Sephadex G-100. Symbols: intact pine C-I-M (a), microwave irradiated at 206°C (b), 215°C (c), 224°C (d), and 237°C (e). The arrows indicate the positions of void volume.

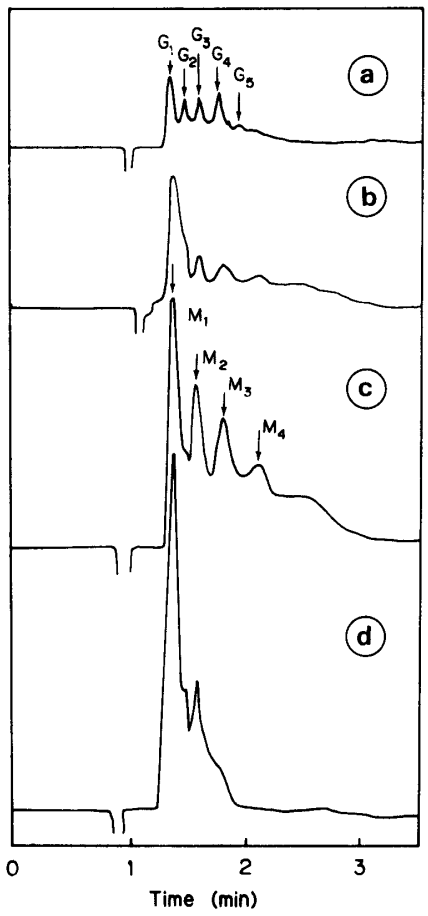

Fig. 3. High performance liquid chromatography of the neutral water-soluble components. Symbols: microwave irradiated to 183°C (a), 215°C (b), 224°C (c), and 237°C (d); G_1–G_4, eluting positions of glucose and cello-oligosaccharides having d.p. of 2–4; M_1–M_4, eluting positions of mannose and β-(1→4)-linked manno-oligosaccharides.

bondings in C-I-M could be splitted during microwave irradiation above this temperature. The mean weight average molecular weight distribution of the carbohydrate moieties was estimated as shown in Table 1, which indicated that the carbohydrate portion of C-I-M was gradually depolymerized into oligosaccharides and monosaccharides by the microwave irradiation. Hence, we analyzed the oligosaccharide composition by HPLC and TLC. A mixtures of glucomanno-oligosaccharides having d.p. of 2–5 and monosaccharides could be identified by HPLC (Fig. 3). It was found that the formation of cello-oligosaccharides predominated below 210°C, above which, however, manno-oligosaccharides predominated. These results were confirmed by TLC (data not shown). The monosaccharide compositional analysis of the water-soluble components by GLC (Table 2) indicates that the amount of glucose increased with increasing temperature, while the reverse was observed

Table 2. Neutral monosaccharides produced by microwave irradiation of pine C-I-M

Samples	Temperature (°C)	Neutral monosaccharides[a]			Yield[b] (%)
		Man	Gal	Glc	
C-I-M in water	207	50.5	34.8	14.7	2.2
	216	49.8	30.2	20.0	5.2
	225	61.0	10.4	28.5	6.8
	237	60.0	8.7	31.3	16.2
C-I-M in 0.5% acetic acid	200	54.0	12.7	33.3	12.4
	212	55.7	10.0	34.3	29.7
	223	53.1	7.0	39.9	28.8
	229	52.4	4.9	42.6	16.1

a) Values are expressed as a percentage of the total, neutral sugars.
b) Based on the dry matter of the original C-I-M.

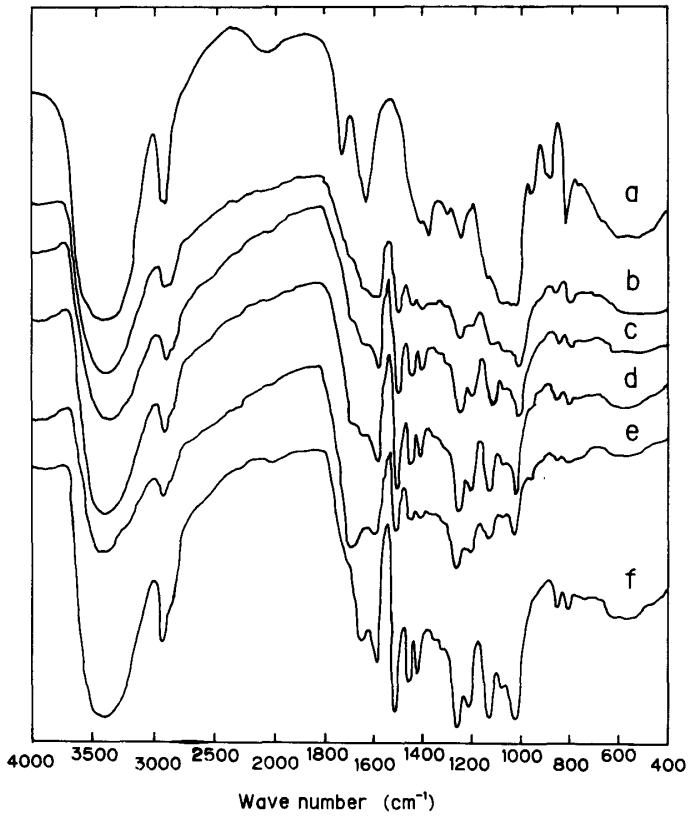

Fig. 4. Infra red spectra of the precipitates formed during microwave irradiation, intact pine C-I-M and milled wood lignin. Symbols: intact pine C-I-M (a); microwave irradiated at 206°C (b), 215°C (c), 225°C (d), and 237°C (e); and pine milled wood lignin (f).

in that of galactose. Based on the present results, the partially acetylated galactoglucomannan in C-I-M was found to be autohydrolyzed by catalytic reactions of acetic acid released from itself during microwave irradiation.

The formation of the water-insoluble materials initiated at 200°C, above which it increased with increasing temperature to attain a maximum value of 8.4% at 237°C (Fig. 1). The carbohydrate content in the precipitated materials was initially high (23.0% at 207°C), but rapidly decreased with increasing temperature to 0.1% at 237°C (Table 1). Since the carbohydrates remained in the precipitates seem to contain residues linking to lignin, the present results suggest that the linkages between lignin and carbohydrate are splittable by microwave irradiation. Assuming that whole lignin in C-I-M (6.9%) became insoluble, the total amount of the precipitate at 237°C were summed up to 7.0%, which was about 1.4% lower than the observed value. This may indicate that the secondary degradation products of lignin and/or carbohydrate are contained in the precipitate. For analysis of the structure of the precipitates, IR spectra of the precipitates were measured (Fig. 4). The spectra of the precipitates did not show any clear bands due to galactoglucomannan but had well-defined bands ascribable to the typical softwood lignin[11,12]. The outstanding difference between the spectra of the precipitates and MWL was in that the former had two additional bands at 1700–1705 cm^{-1} and

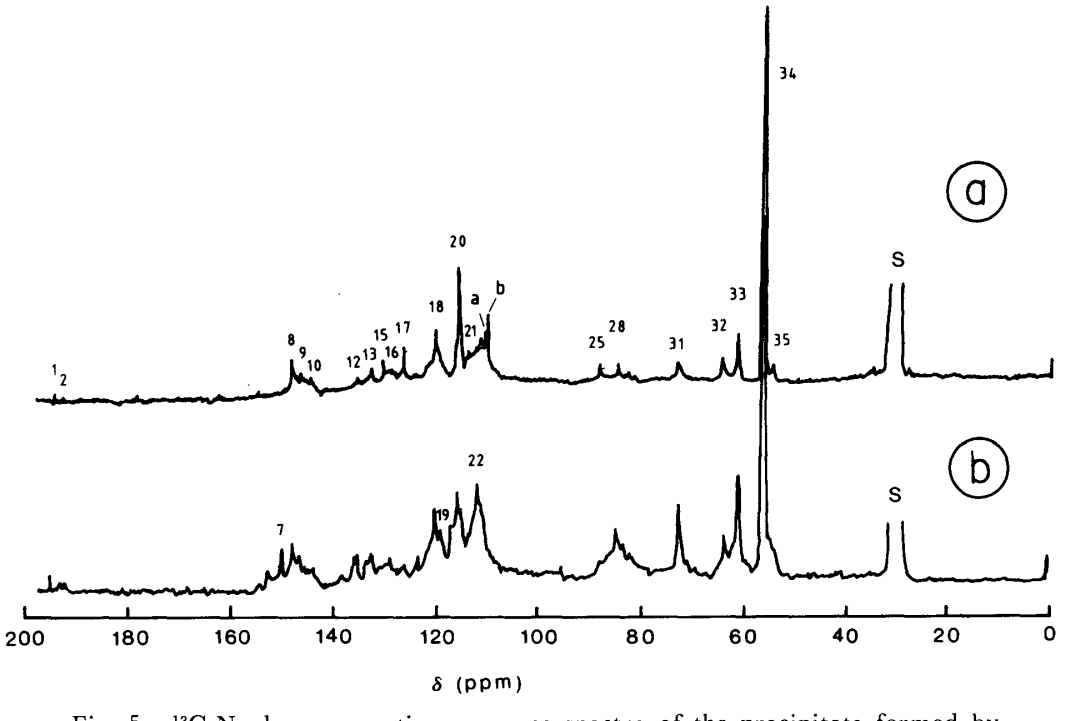

Fig. 5. ^{13}C-Nuclear magnetic resonance spectra of the precipitate formed by microwave irradiation of pine C-I-M to 237°C (a), and pine milled wood lignin (b). Symbol: solvent (s).

970 cm^{-1}. The intensity of these bands became higher with increase in heating temperature. These two bands were assigned[11] to unconjugated carbonyl groups (1700–1705 cm^{-1}), and α,β-unsaturation and α-carbonyl groups (970 cm^{-1}), respectively. The structure of the precipitates was further analyzed by ^{13}C-NMR spectroscopy. A representative spectrum of the precipitate formed at 237°C is shown in Fig.5 together with that of the MWL of Akamatsu for comparison. The signals were assigned as listed in Table 3 based on the literature data[13~17]. The spectra of the precipitates had characteristic peaks similar to MWL. However, as the temperature increased above 200°C, the β-*O*-4 structure was gradually degraded, while

Table 3. Assignments of the ^{13}C-nuclear magnetic resonance spectrum of the precipitate formed during microwave irradiation of pine C-I-M to 237°C

Signal No.[a]	δ (ppm)	Assignment[b]
1	194.7	α-C=O and γ-CHO in cinnamaldehyde
2	191.0	α-CHO
7	150.6	G4 (etherified)
8	148.2	G3, C-4 in β-5
9	146.7	G4 (non-etherified), C-4 in G-CH=CH— and G-CHOH—
10	144.5	C-4 in β-5 and β-β
12	136.3	C-1 in β-*O*-4 and β-β (etherfied)
13	133.8	C-1 in β-*O*-4 and β-β (non-etherified)
15	130.8	C-1 in G-CH=CH-, C-1/5 in β-5
16	129.7	G1, C-2/6 in *p*-hydroxyphenyl
17	126.8	G6 (α-C=O), C-1 in β-β
18	120.3	C-6 in β-*O*-4 (G-CHOH—) and G-CH=CH—
19	117.5	C-5 in β-*O*-4 (etherified), C-6 in β-5
20	115.7	C-5 in β-*O*-4, C-3/5 in *p*-hydroxyphenyl
21	113.8	C-2 in G-C=O, G-CH_2— and G-CH—, C-2 in β-5
22	112.3	C-2 in β-*O*-4 (general and G-CHOH—)
a)	110.6	C-2 in β-5 and G-CH=CH—
b)	110.0	C-2 in 5-5 and 5-*O*-4
25	88.1	C-α in β-5
28	85.2	C-β in β-*O*-4, C-α in alkyl ethers
31	73.4	C-α in β-*O*-4, C-γ in β-β, C-γ in alkyl ethers
32	64.0	C-β and γ in β-1, C-γ in β-5 and G-CH=CH—CH_2OH
33	61.7	C-γ in β-*O*-4 and G-CH_2—CH_2—CH_2OH
34	56.3	OCH_3
35	54.6	C-β in β-β and β-5

Symbol: G, Guaiacyl.

a) Nomenclature specified in Fig. 5 except a and b was done according to Lüdemann and Nimz[13].

b) Assignments were made according to the literature data (Lüdemann and Nimz[13], Nimz *et al*[14], Kringstad and Mörck[15], and Hemmingson[16]).

the condensed type structures such as β-β and β-5 remained. The modification of the side-chain of lignin was also noted in the precipitate, confirming the results of IR spectra. Similar IR and NMR spectroscopic results have been reported with steam-exploded pine lignin[16]. Both the microwave irradiation and steam-explosion processes are likely to involve similar degradation mechanisms. The present results clearly indicate that lignin as well as galactoglucomannan in C-I-M was decomposed by microwave irradiation.

3.2 Effects of addition of acetic acid during microwave irradiation

It has previously been shown that the presence of 0.5% acetic acid during microwave irradiation enhanced degradation of cellulose and further increased the rate of its enzymatic saccharification[5]. However, the effect of acetic acid on hemicellulose has not been clarified yet. For the initial approach to solve this problem, C-I-M was heated by microwave irradiation in the presence of 0.5% acetic acid. As shown in Fig. 1, the degradation of galactoglucomannan specified by measuring the reducing sugar production was facilitated by a factor of 4.4–6.8 at 200–210°C by the addition of acetic acid. The reducing sugar production showed a maximum (74.2%) at about 210°C, in contrast to the case without addition of acetic acid. This temperature was about 20°C lower than that with the native sapwood of Akamatsu. These results clearly indicate the importance of acetic acid concentration for degradation of hemicellulose during microwave irradiation. The production of neutral monosaccharides also markedly increased by the addition of 0.5% acetic acid (Table 2). The temperature dependency of the amounts of the free galactose and glucose residues was similar to that observed without addition of acetic acid. The amount of the water-insoluble component increased with increase in the temperature range from 200°C to 230°C and attained to 9.2% at 229°C which was slightly higher than the value (8.4%) obtained without addition of acetic acid at 237°C. In conclusion, acetic acid was established to be an excellent reagent for enhancement of the extent of depolymerization of galactoglucomannan in C-I-M.

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References

1) J. Azuma, F. Tanaka and T. Koshijima: *Mokuzai Gakkaishi*, **30**, 501 (1984).

2) J. AZUMA, F. TANAKA and T. KOSHIJIMA: *J. Ferment. Technol.*, **62**, 377 (1984).
3) J. AZUMA and T. KOSHIJIMA: *Mokuzai Kenkyu・Shiryo* (Japanese), **20**, 22 (1985).
4) J. AZUMA, F. TANAKA and T. KOSHIJIMA: *Wood Res.*, **71**, 13 (1985).
5) J. AZUMA, T. ASAI, M. ISAKA and T. KOSHIJIMA: *J. Ferment. Technol.*, **63**, 529 (1985).
6) J. AZUMA, A. OZAKI and T. KOSHIJIMA: *Mokuzai Kenkyu・Shiryo* (Japanese), **21**, 78 (1985).
7) T. KOSHIJIMA, T. TANIGUCHI and R. TANAKA: *Holzforschung*, **26**, 211 (1972).
8) A. BJÖRKMAN: *Svensk Papperstidn*, **60**, 243 (1957).
9) A. BJÖRKMAN: *Svensk Papperstidn*, **59**, 477 (1956).
10) G.L. MILLER: *Anal. Chem.*, **31**, 426 (1959).
11) H.L. HERGERT: Lignins, K.V. Sarkanen and C.H. Ludwig (eds), Wiley-Interscience, N.Y., p.267 (1971).
12) K. KAWAMURA and T. HIGUCHI: *Mokuzai Gakkaishi*, **10**, 200 (1964).
13) H.-D. LÜDEMANN and H.H. NIMZ: *Makromol. Chem.*, **175**, 2409 (1974).
14) H.H. NIMZ, D. ROBERT, O. FAIX and M. NEMR: *Holzforschung*, **35**, 16 (1981).
15) K.P. KRINGSTAD and R. MÖRCK: *Holzforschung*, **37**, 237 (1983).
16) J.A. HEMMINGSON: *J. Wood Chem. Technol.*, **3**, 289 (1983).